

HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE

This application claims benefit of U.S. Provisional Patent Application
5 Serial No. 60/103,050, filed October 5, 1998.

FIELD OF THE INVENTION

The present invention relates to active fragments of a hypersensitive
10 response elicitor which fragments do not elicit a hypersensitive response.

BACKGROUND OF THE INVENTION

Interactions between bacterial pathogens and their plant hosts generally
15 fall into two categories: (1) compatible (pathogen-host), leading to intercellular
bacterial growth, symptom development, and disease development in the host plant;
and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a
particular type of incompatible interaction occurring, without progressive disease
symptoms. During compatible interactions on host plants, bacterial populations
20 increase dramatically and progressive symptoms occur. During incompatible
interactions, bacterial populations do not increase, and progressive symptoms do not
occur.

The hypersensitive response is a rapid, localized necrosis that is
associated with the active defense of plants against many pathogens (Kiraly, Z.,
25 "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant
Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed.
Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177
in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic
Press, New York (1982)). The hypersensitive response elicited by bacteria is readily
30 observed as a tissue collapse if high concentrations ($\geq 10^7$ cells/ml) of a limited
host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated
into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower
levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of
Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

“Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf,”
Phytopathology 54:474-477 (1963); Turner, et al., “The Quantitative Relation
Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction,”
Phytopathology 64:885-890 (1974); Klement, Z., “Hypersensitivity,” pages 149-177
5 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic
Press, New York (1982)). The capacities to elicit the hypersensitive response in a
nonhost and be pathogenic in a host appear linked. As noted by Klement, Z.,
“Hypersensitivity,” pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S.
Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause
10 physiologically similar, albeit delayed, necroses in their interactions with compatible
hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis
is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., “Gene
Cluster of *Pseudomonas syringae* pv. ‘phaseolicola’ Controls Pathogenicity of Bean
Plants and Hypersensitivity on Nonhost Plants,” J. Bacteriol. 168:512-22 (1986);
15 Willis, D.K., et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe
Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold
clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in Gram-negative plant pathogens,
where they are clustered, conserved, and in some cases interchangeable (Willis, D.K.,
20 et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe Interact. 4:132-
138 (1991); Bonas, U., “*hrp* Genes of Phytopathogenic Bacteria,” pages 79-98 in:
Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants
and Animals - Molecular and Cellular Mechanisms, J.L. Dangel, ed. Springer-Verlag,
Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway
25 similar to one used by *Yersinia*, *Shigella*, and *Salmonella* spp. to secrete proteins
essential in animal diseases (Van Gijsegem, et al., “Evolutionary Conservation of
Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria,” Trends
Microbiol. 1:175-180 (1993)). In *E. amylovora*, *P. syringae*, and *P. solanacearum*,
hrp genes have been shown to control the production and secretion of glycine-rich,
30 protein elicitors of the hypersensitive response (He, S.Y., et al. “*Pseudomonas*
Syringae pv. *Syringae* HarpinPss: a Protein that is Secreted via the Hrp Pathway and
Elicits the Hypersensitive Response in Plants,” Cell 73:1255-1266 (1993), Wei, Z.-H.,

et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al.

"PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)). However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among Gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "*Erwinia chrysanthemi* Harpin_{Ech}: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA⁻ Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

The present invention seeks to identify fragments of hypersensitive response elicitor proteins or polypeptides, which fragments do not elicit a hypersensitive response but are active when utilized in conjunction with plants.

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SUMMARY OF THE INVENTION

The present invention is directed to isolated fragments of an *Erwinia* hypersensitive response elicitor protein or polypeptide which fragments do not elicit a hypersensitive response in plants but are otherwise active when utilized in
10 conjunction with plants. Also disclosed are isolated DNA molecules which encode such fragments.

The fragments of hypersensitive response elicitors according to the present invention have the following activity when utilized in conjunction with plants: imparting disease resistance to plants, enhancing plant growth and/or controlling
15 insects. This involves applying the fragments in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

As an alternative to applying the fragments to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic
20 plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor protein or polypeptide in accordance with the present invention and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control
25 insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding such a fragment can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows truncated proteins of the hypersensitive response elicitor protein or polypeptide.

Figure 2 shows a list of synthesized oligonucleotide primers for construction of truncated harpin proteins. N represents the N-terminus (5' region), and C represents the C-terminus (3' region). The primers correspond to the indicated sequence identification numbers for the present application: N1 (SEQ. ID. No. 1), N76 (SEQ. ID. No. 2), N99 (SEQ. ID. No. 3), N105 (SEQ. ID. No. 4), N110 (SEQ. ID. No. 5), N137 (SEQ. ID. No. 6), N150 (SEQ. ID. No. 7), N169 (SEQ. ID. No. 8), N210 (SEQ. ID. No. 9), N267 (SEQ. ID. No. 10), N343 (SEQ. ID. No. 11), C75 (SEQ. ID. No. 12), C104 (SEQ. ID. No. 13), C168 (SEQ. ID. No. 14), C180 (SEQ. ID. No. 15), C204 (SEQ. ID. No. 16), C209 (SEQ. ID. No. 17), C266 (SEQ. ID. No. 18), C342 (SEQ. ID. No. 19), and C403 (SEQ. ID. No. 20).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to isolated fragments of a hypersensitive response elicitor protein or polypeptide where the fragments do not elicit a hypersensitive response but have other activity in plants. Also disclosed are DNA molecules encoding such fragments as well as expression systems, host cells, and plants containing such molecules. Uses of the fragments themselves and the DNA molecules encoding them are disclosed.

The fragments of hypersensitive response elicitor polypeptides or proteins according to the present invention are derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 21 as follows:

Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
1 5 10 15

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
20 25 30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
35 40 45

Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
50 55 60

Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
65 70 75 80

Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
85 90 95

Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
100 105 110

Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
115 120 125

Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
130 135 140

Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145 150 155 160

Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
165 170 175

Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
180 185 190

Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
195 200 205

Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
210 215 220

Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225 230 235 240

Sub
A2
Cont'd

Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
245 250 255
Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
260 265 270
Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
275 280 285
Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
290 295 300
Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
305 310 315 320
Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
325 330 335
Asn Ala

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This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 22 as follows:

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CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTTCGA CACCGTTACG 60
GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTCGCCGCA ATCCGGCGTC 120
GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAACCTCA TGATGCAGAT TCAGCCGGGG 180
25 CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCGGCAGAG 240
TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG 300
CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTTGAACT GGCGGGAATG 360
ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC 420
CGATCATTAA GATAAAGGCG GCTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT 480
30 CACCGTCGGC GTCACTCAGT AACAAGTATC CATCATGATG CCTACATCGG GATCGGCGTG 540
GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA 600
AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC 660
TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCCAGCG TGGATAAACT 720
GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT 780
35 GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC 840

Sub A2 Control

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TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA 900

TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC 960

CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCATG CTGAACGCCA GCCAGATGAC 1020

CCAGGGTAAT ATGAATGCGT TCGGCGAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG 1080

CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT 1140

GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT 1200

GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA 1260

CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA 1320

TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA 1380

GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG 1440

CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA 1500

TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC 1560

GGCTGTCTGC GCGGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA 1620

ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC 1680

TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA 1740

ACGCACATTT TCCGTTTCAT TCGCGTCGTT ACGCGGCACA ATCGCGATGG CATCTTCCTC 1800

GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC 1860

CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCCTTTAG 1920

CAGATAGATT GCGGTTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG 1980

GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC 2040

AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG 2100

GTTGCTCATC ATCTTTCTCC ATCTGGGCGA CCTGATCGGT T 2141

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 23 as follows:

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
1 5 10 15

Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
20 25 30

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[illegible]

340 345 350

Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
355 360 365

Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
370 375 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
385 390 395 400

Gly Ala Ala

This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 24 as follows:

20 AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGCGAGGG TACGTTTGAA TTATTCATAA 60
GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT 120
ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG 180
GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG 240
GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG 300
25 GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA 360
GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTTCGC TGAACACGCT GGGCTCGAAA 420
GGCGGCAACA ATACCACTTC AACAACAAAT TCCCCTGCTG ACCAGGCGCT GGGTATTAAC 480
TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC 540
CCGATGCAGC AGCTGCTGAA GATGTTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG 600
30 CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC 660
GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG 720
CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC 780
GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGA CTACCAGCAG 840

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A3.1
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TTAGGTAACG	CCGTGGGTAC	CGGTATCGGT	ATGAAAGCGG	GCATTCAGGC	GCTGAATGAT	900
ATCGGTACGC	ACAGGCACAG	TTCAACCCGT	TCTTTCGTCA	ATAAAGGCGA	TCGGGCGATG	960
GCGAAGGAAA	TCGGTCAGTT	CATGGACCAG	TATCCTGAGG	TGTTTGCCAA	GCCGCAGTAC	1020
CAGAAAGGCC	CGGGTCAGGA	GGTGAAAACC	GATGACAAAT	CATGGGCAAA	AGCACTGAGC	1080
AAGCCAGATG	ACGACGGAAT	GACACCAGCC	AGTATGGAGC	AGTTCAACAA	AGCCAAGGGC	1140
ATGATCAAAA	GGCCCATGGC	GGGTGATACC	GGCAACGGCA	ACCTGCAGGC	ACGCGGTGCC	1200
GGTGGTTCTT	CGCTGGGTAT	TGATGCCATG	ATGGCCGGTG	ATGCCATTAA	CAATATGGCA	1260
CTTGGAAGC	TGGGCGCGGC	TTAAGCTT				1288

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Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 25 as follows:

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ATGTCAATTC	TTACGCTTAA	CAACAATACC	TCGTCCTCGC	CGGGTCTGTT	CCAGTCCGGG	60
GGGGACAACG	GGCTTGGTGG	TCATAATGCA	AATTCTGCGT	TGGGGCAACA	ACCCATCGAT	120
CGGCAAACCA	TTGAGCAAAT	GGCTCAATTA	TTGGCGGAAC	TGTTAAAGTC	ACTGCTATCG	180
CCACAATCAG	GTAATGCGGC	AACCGGAGCC	GGTGGCAATG	ACCAGACTAC	AGGAGTTGGT	240
AACGCTGGCG	GCCTGAACGG	ACGAAAAGGC	ACAGCAGGAA	CCACTCCGCA	GTCTGACAGT	300
CAGAACATGC	TGAGTGAGAT	GGGCAACAAC	GGGCTGGATC	AGGCCATCAC	GCCCGATGGC	360
CAGGGCGGCG	GGCAGATCGG	CGATAATCCT	TTACTGAAAG	CCATGCTGAA	GCTTATTGCA	420
CGCATGATGG	ACGGCCAAAG	CGATCAGTTT	GGCCAACCTG	GTACGGGCAA	CAACAGTGCC	480
TCTTCCGGTA	CTTCTTCATC	TGGCGGTTCC	CCTTTTAAAG	ATCTATCAGG	GGGGAAGGCC	540
CCTTCCGGCA	ACTCCCCTTC	CGGCAACTAC	TCTCCCGTCA	GTACCTTCTC	ACCCCCATCC	600
ACGCCAACGT	CCCCTACCTC	ACCGCTTGAT	TTCCCTTCTT	CTCCCACCAA	AGCAGCCGGG	660
GGCAGCACGC	CGGTAACCGA	TCATCCTGAC	CCTGTTGGTA	GCGCGGGCAT	CGGGGCCGGA	720
AATTCGGTGG	CCTTCACCAG	CGCCGGCGCT	AATCAGACGG	TGCTGCATGA	CACCATTACC	780
GTGAAAGCGG	GTCAGGTGTT	TGATGGCAAA	GGACAAACCT	TCACCGCCGG	TTCAGAATTA	840
GGCGATGGCG	GCCAGTCTGA	AAACCAGAAA	CCGCTGTTTA	TACTGGAAGA	CGGTGCCAGC	900
CTGAAAAACG	TCACCATGGG	CGACGACGGG	GCGGATGGTA	TTCATCTTTA	CGGTGATGCC	960
AAAATAGACA	ATCTGCACGT	CACCAACGTG	GGTGAGGACG	CGATTACCGT	TAAGCCAAAC	1020
AGCGCGGGCA	AAAAATCCCA	CGTTGAAATC	ACTAACAGTT	CCTTCGAGCA	CGCCTCTGAC	1080
AAGATCCTGC	AGCTGAATGC	CGATACTAAC	CTGAGCGTTG	ACAACGTGAA	GGCCAAAGAC	1140

TTTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC 1200
CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTTAAAA GCGATAGCGA GGGGCTAAAC 1260
GTCAATACCA GTGATATCTC ACTGGGTGAT GTTGAAAACC ACTACAAAGT GCCGATGTCC 1320
GCCAACCTGA AGGTGGCTGA ATGA 1344

See GenBank Accession No. U94513. The isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 26 as follows:

Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
1 5 10 15
Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
20 25 30
Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
35 40 45
Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
50 55 60
Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
65 70 75 80
Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
85 90 95
Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
100 105 110
Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
115 120 125
Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
130 135 140
Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
145 150 155 160
Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
165 170 175
Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
180 185 190
Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro
195 200 205
Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
210 215 220

5 Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly
225 230 235 240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
245 250 255

10 Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln
260 265 270

Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
275 280 285

15 Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val
290 295 300

Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala
305 310 315 320

20 Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr
325 330 335

Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn
340 345 350

25 Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
355 360 365

Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
370 375 380

30 Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
385 390 395 400

35 His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
405 410 415

Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu
420 425 430

40 Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu
435 440 445

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663 which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 27 as follows:

	ATGGAATTAA	AATCACTGGG	AACTGAACAC	AAGGCGGCAG	TACACACAGC	GGCGCACAAAC	60
	CCTGTGGGGC	ATGGTGTTC	CTTACAGCAG	GGCAGCAGCA	GCAGCAGCCC	GCAAAATGCC	120
5	GCTGCATCAT	TGGCGGCAGA	AGGCAAAAAT	CGTGGGAAAA	TGCCGAGAAT	TCACCAGCCA	180
	TCTACTGCGG	CTGATGGTAT	CAGCGCTGCT	CACCAGCAAA	AGAAATCCTT	CAGTCTCAGG	240
10	GGCTGTTTGG	GGACGAAAAA	ATTTTCCAGA	TCGGCACCGC	AGGGCCAGCC	AGGTACCACC	300
	CACAGCAAAG	GGGCAACATT	GCGCGATCTG	CTGGCGCGGG	ACGACGGCGA	AACGCAGCAT	360
	GAGGCGGCCG	CGCCAGATGC	GGCGCGTTTG	ACCCGTTTCG	GCGGCGTCAA	ACGCCGCAAT	420
15	ATGGACGACA	TGGCCGGGCG	GCCAATGGTG	AAAGGTGGCA	GCGGCGAAGA	TAAGGTACCA	480
	ACGCAGCAAA	AACGGCATCA	GCTGAACAAT	TTTGCCAGA	TGCGCCAAAC	GATGTTGAGC	540
20	AAAATGGCTC	ACCCGGCTTC	AGCCAACGCC	GGCGATCGCC	TGCAGCATTC	ACGCCCGCAC	600
	ATCCCGGGTA	GCCACCACGA	AATCAAGGAA	GAACCGGTTG	GCTCCACCAG	CAAGGCAACA	660
	ACGGCCACG	CAGACAGAGT	GGAAATCGCT	CAGGAAGATG	ACGACAGCGA	ATTCCAGCAA	720
25	CTGCATCAAC	AGCGGCTGGC	GCGCGAACGG	GAAAATCCAC	CGCAGCCGCC	CAAACCTCGGC	780
	GTTGCCACAC	CGATTAGCGC	CAGGTTTCAG	CCCAAATGA	CTGCGGTTGC	GGAAAGCGTC	840
30	CTTGAGGGGA	CAGATACCAC	GCAGTCACCC	CTTAAGCCGC	AATCAATGCT	GAAAGGAAGT	900
	GGAGCCGGGG	TAACGCCGCT	GGCGGTAACG	CTGGATAAAG	GCAAGTTGCA	GCTGGCACCG	960
	GATAATCCAC	CCGCGCTCAA	TACGTTGTTG	AAGCAGACAT	TGGGTAAAGA	CACCCAGCAC	1020
35	TATCTGGCGC	ACCATGCCAG	CAGCGACGGT	AGCCAGCATC	TGCTGTGGA	CAACAAAGGC	1080
	CACCTGTTTG	ATATCAAAAG	CACCGCCACC	AGCTATAGCG	TGCTGCACAA	CAGCCACCCC	1140
40	GGTGAGATAA	AGGGCAAGCT	GGCGCAGGCG	GGTACTGGCT	CCGTCAGCGT	AGACGGTAAA	1200
	AGCGGCAAGA	TCTCGCTGGG	GAGCGGTACG	CAAAGTCACA	ACAAAACAAT	GCTAAGCCAA	1260
	CCGGGGGAAG	CGCACCGTTC	CTTATTAACC	GGCATTTGGC	AGCATCCTGC	TGGCGCAGCG	1320
45	CGGCCGCAGG	GCGAGTCAAT	CCGCCTGCAT	GACGACAAAA	TTCATATCCT	GCATCCGGAG	1380
	CTGGGCGTAT	GGCAATCTGC	GGATAAAGAT	ACCCACAGCC	AGCTGTCTCG	CCAGGCAGAC	1440
50	GGTAAGCTCT	ATGCGCTGAA	AGACAACCGT	ACCTGCAAA	ACCTCTCCGA	TAATAAATCC	1500
	TCAGAAAAGC	TGGTCGATAA	AATCAAATCG	TATTCCGTTG	ATCAGCGGGG	GCAGGTGGCG	1560
	ATCCTGACGG	ATACTCCCGG	CCGCCATAAG	ATGAGTATTA	TGCCCTCGCT	GGATGCTTCC	1620
55	CCGGAGAGCC	ATATTTCCCT	CAGCCTGCAT	TTTGCCGATG	CCCACCAGGG	GTTATTGCAC	1680
	GGGAAGTCGG	AGCTTGAGGC	ACAATCTGTC	GCGATCAGCC	ATGGGCGACT	GGTTGTGGCC	1740
60	GATAGCGAAG	GCAAGCTGTT	TAGCGCCGCC	ATCCGAAGC	AAGGGGATGG	AAACGAACTG	1800
	AAAATGAAAG	CCATGCCTCA	GCATGCGCTC	GATGAACATT	TTGGTCATGA	CCACCAGATT	1860
	TCTGGATTTT	TCCATGACGA	CCACGGCCAG	CTTAATGCGC	TGGTGAAAAA	TAACCTCAGG	1920
65	CAGCAGCATG	CCTGCCCGTT	GGGTAACGAT	CATCAGTTTC	ACCCCGGCTG	GAACCTGACT	1980

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CGTGTGGGGC

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GATGCGCTGG TTATCGACAA TCAGCTGGGG CTGCATCATA CCAATCCTGA ACCGCATGAG 2040
ATTCTTGATA TGGGGCATT TGGGGCATT AGGCAGCCTG GCGTTACAGG AGGGCAAGCT TCACATATTTT 2100
GACCAGCTGA CCAAAGGGTG GACTGGCGCG GAGTCAGATT GTAAGCAGCT GAAAAAAGGC 2160
CTGGATGGAG CAGCTTATCT ACTGAAAGAC GGTGAAGTGA AACGCCTGAA TATTAATCAG 2220
AGCACCTCCT CTATCAAGCA CGGAACGGAA AACGTTTTTT CGCTGCCGCA TGTGCGCAAT 2280
AAACCGGAGC CGGGAGATGC CCTGCAAGGG CTGAATAAAG ACGATAAGGC CCAGGCCATG 2340
GCGGTGATTG GGGTAAATAA ATACCTGGCG CTGACGGAAA AAGGGGACAT TCGCTCCTTC 2400
CAGATAAAAC CCGGCACCCA GCAGTTGGAG CGGCCGGCAC AAATCTCAG CCGCGAAGGT 2460
ATCAGCGGCG AACTGAAAGA CATTCATGTC GACCACAAGC AGAACCTGTA TGCCTTGACC 2520
CACGAGGGAG AGGTGTTTCA TCAGCCGCGT GAAGCCTGGC AGAATGGTGC CGAAAGCAGC 2580
AGCTGGCACA AACTGGCGTT GCCACAGAGT GAAAGTAAGC TAAAAAGTCT GGACATGAGC 2640
CATGAGCACA AACCGATTGC CACCTTTGAA GACGGTAGCC AGCATCAGCT GAAGGCTGGC 2700
GGCTGGCACG CCTATGCGGC ACCTGAACGC GGGCCGCTGG CGGTGGGTAC CAGCGGTTCA 2760
CAAACCGTCT TTAACCGACT AATGCAGGGG GTGAAAGGCA AGGTGATCCC AGGCAGCGGG 2820
TTGACGGTTA AGCTCTCGGC TCAGACGGGG GGAATGACCG GCGCCGAAGG GCGCAAGGTC 2880
AGCAGTAAAT TTTCCGAAAG GATCCGCGCC TATGCGTTCA ACCCAACAAT GTCCACGCCG 2940
CGACCGATTA AAAATGCTGC TTATGCCACA CAGCACGGCT GGCAGGGGCG TGAGGGGTTG 3000
AAGCCGTTGT ACGAGATGCA GGGAGCGCTG ATTAAACAAC TGGATGCGCA TAACGTTCTG 3060
CATAACGCGC CACAGCCAGA TTTGCAGAGC AAATGGAAA CTCTGGATTT AGGCGAACAT 3120
GGCGCAGAAT TGCTTAACGA CATGAAGCGC TTCCGCGACG AACTGGAGCA GAGTGCAACC 3180
CGTTCGGTGA CCGTTTTAGG TCAACATCAG GGAGTGCTAA AAAGCAACGG TGAAATCAAT 3240
AGCGAATTTA AGCCATCGCC CGGCAAGGCG TTGGTCCAGA GCTTTAACGT CAATCGCTCT 3300
GGTCAGGATC TAAGCAAGTC ACTGCAACAG GCAGTACATG CCACGCCGCC ATCCGCAGAG 3360
AGTAACTGC AATCCATGCT GGGGCACTTT GTCAGTGCCG GGGTGGATAT GAGTCATCAG 3420
AAGGGCGAGA TCCCCTGGG CCGCCAGCGC GATCCGAATG ATAAAACCGC ACTGACCAAA 3480
TCGCGTTTAA TTTTAGATAC CGTGACCATC GGTGAACTGC ATGAACTGGC CGATAAGGCG 3540
AAACTGGTAT CTGACCATAA ACCCGATGCC GATCAGATAA AACAGCTGCG CCAGCAGTTC 3600
GATACGCTGC GTGAAAAGCG GTATGAGAGC AATCCGGTGA AGCATTACAC CGATATGGGC 3660
TTCACCCATA ATAAGGCGCT GGAAGCAAAC TATGATGCGG TCAAAGCCTT TATCAATGCC 3720
TTTAAGAAAG AGCACCACGG CGTCAATCTG ACCACGCGTA CCGTACTGGA ATCACAGGGC 3780
AGTGCAGGAGC TGGCGAAGAA GCTCAAGAAT ACGTGTTGT CCCTGGACAG TGGTGAAAGT 3840
ATGAGCTTCA GCCGGTCATA TGGCGGGGGC GTCAGCACTG TCTTTGTGCC TACCCTTAGC 3900

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AAGAAGGTGC CAGTTCCGGT GATCCCCGGA GCCGGCATCA CGCTGGATCG CGCCTATAAC 3960
CTGAGCTTCA GTCGTACCAG CGGCGGATTG AACGTCAGTT TTGGCCGCGA CGGCGGGGTG 4020
AGTGGTAACA TCATGGTCGC TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA 4080
ACCAGTGCAG GTAACGCCAG TGACTGGTTG AGCGCAAAAC ATAAAATCAG CCCGGACTTG 4140
CGTATCGGCG CTGCTGTGAG TGGCACCCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT 4200
AAGCTGACAG AGGATGAGCT GCCTGGCTTT ATCCATGGCT TGACGCATGG CACGTTGACC 4260
CCGGCAGAAC TGTTGCAAAA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAAACTGACG 4320
TTTAGCGTCG ATACCTCGGC AAATCTGGAT CTGCGTGCCG GTATCAATCT GAACGAAGAC 4380
GGCAGTAAAC CAAATGGTGT CACTGCCCGT GTTCTGCCG GGCTAAGTGC ATCGGCAAAC 4440
CTGGCCGCCG GCTCGCGTGA ACGCAGCACC ACCTCTGGCC AGTTTGGCAG CACGACTTCG 4500
GCCAGCAATA ACCGCCCAAC CTTCTCAAC GGGGTCGGCG CGGGTGCTAA CCTGACGGCT 4560
GCTTTAGGGG TTGCCCATTC ATCTACGCAT GAAGGGAAAC CGGTCTGGGAT CTTCCCGGCA 4620
TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC 4680
AGCCTGGAAT TGAAGCGCGC GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC 4740
ACGCTGGGAA AACACTTTAA GGATAGCGCC ACAACGAAGA TGCTTGCCGC TCTCAAAGAG 4800
TTAGATGACG CTAAGCCCGC TGAACAACTG CATATTTTAC AGCAGCATTT CAGTGCAAAA 4860
GATGTCGTCG GTGATGAACG CTACGAGGCG GTGCGCAACC TGAAAAAACT GGTGATACGT 4920
CAACAGGCTG CGGACAGCCA CAGCATGGAA TTAGGATCTG CCAGTCACAG CACGACCTAC 4980
AATAATCTGT CGAGAATAAA TAATGACGGC ATTGTGAGC TGCTACACAA ACATTTTCGAT 5040
GCGGCATTAC CAGCAAGCAG TGCCAAACGT CTTGGTGAAA TGATGAATAA CGATCCGGCA 5100
CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCA GCAGCGCCAG CGTGTCGATG 5160
GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAAGCAA TACTGGACGG TAAGGTCGGT 5220
CGTGAAGAAG TGGGAGTACT TTTCCAGGAT CGTAACAACT TCGGTGTTAA ATCGGTCAGC 5280
GTCAGTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG 5340
AGCAACAGCG CTGCTATGAG CATGGAGCGC AACATCGGAA CCATTAATTT TAAATACGGC 5400
CAGGATCAGA ACACCCACG GCGATTTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG 5460
CAGGTCGCAT CTGCGCTTAC TGATTTGAAG AAGGAAGGGC TGGAAATGAA GAGCTAA 5517

This DNA molecule is known as the dspE gene for *Erwinia amylovora*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 28 as follows:

2nd
AS

	Met	Glu	Leu	Lys	Ser	Leu	Gly	Thr	Glu	His	Lys	Ala	Ala	Val	His	Thr
	1			5					10					15		
5	Ala	Ala	His	Asn	Pro	Val	Gly	His	Gly	Val	Ala	Leu	Gln	Gln	Gly	Ser
				20					25				30			
	Ser	Ser	Ser	Ser	Pro	Gln	Asn	Ala	Ala	Ala	Ser	Leu	Ala	Ala	Glu	Gly
				35				40					45			
10	Lys	Asn	Arg	Gly	Lys	Met	Pro	Arg	Ile	His	Gln	Pro	Ser	Thr	Ala	Ala
	50					55						60				
	Asp	Gly	Ile	Ser	Ala	Ala	His	Gln	Gln	Lys	Lys	Ser	Phe	Ser	Leu	Arg
15	65					70					75					80
	Gly	Cys	Leu	Gly	Thr	Lys	Lys	Phe	Ser	Arg	Ser	Ala	Pro	Gln	Gly	Gln
					85				90					95		
20	Pro	Gly	Thr	Thr	His	Ser	Lys	Gly	Ala	Thr	Leu	Arg	Asp	Leu	Leu	Ala
				100					105					110		
	Arg	Asp	Asp	Gly	Glu	Thr	Gln	His	Glu	Ala	Ala	Ala	Pro	Asp	Ala	Ala
				115				120					125			
25	Arg	Leu	Thr	Arg	Ser	Gly	Gly	Val	Lys	Arg	Arg	Asn	Met	Asp	Asp	Met
	130						135					140				
	Ala	Gly	Arg	Pro	Met	Val	Lys	Gly	Gly	Ser	Gly	Glu	Asp	Lys	Val	Pro
30	145					150					155					160
	Thr	Gln	Gln	Lys	Arg	His	Gln	Leu	Asn	Asn	Phe	Gly	Gln	Met	Arg	Gln
				165					170					175		
35	Thr	Met	Leu	Ser	Lys	Met	Ala	His	Pro	Ala	Ser	Ala	Asn	Ala	Gly	Asp
				180					185					190		
	Arg	Leu	Gln	His	Ser	Pro	Pro	His	Ile	Pro	Gly	Ser	His	His	Glu	Ile
		195						200					205			
40	Lys	Glu	Glu	Pro	Val	Gly	Ser	Thr	Ser	Lys	Ala	Thr	Thr	Ala	His	Ala
	210					215						220				
	Asp	Arg	Val	Glu	Ile	Ala	Gln	Glu	Asp	Asp	Asp	Ser	Glu	Phe	Gln	Gln
45	225					230				235						240
	Leu	His	Gln	Gln	Arg	Leu	Ala	Arg	Glu	Arg	Glu	Asn	Pro	Pro	Gln	Pro
				245					250						255	
50	Pro	Lys	Leu	Gly	Val	Ala	Thr	Pro	Ile	Ser	Ala	Arg	Phe	Gln	Pro	Lys
			260						265					270		
	Leu	Thr	Ala	Val	Ala	Glu	Ser	Val	Leu	Glu	Gly	Thr	Asp	Thr	Thr	Gln
			275					280					285			
55	Ser	Pro	Leu	Lys	Pro	Gln	Ser	Met	Leu	Lys	Gly	Ser	Gly	Ala	Gly	Val
	290					295						300				
	Thr	Pro	Leu	Ala	Val	Thr	Leu	Asp	Lys	Gly	Lys	Leu	Gln	Leu	Ala	Pro
60	305					310					315					320
	Asp	Asn	Pro	Pro	Ala	Leu	Asn	Thr	Leu	Leu	Lys	Gln	Thr	Leu	Gly	Lys
				325					330						335	
65	Asp	Thr	Gln	His	Tyr	Leu	Ala	His	His	Ala	Ser	Ser	Asp	Gly	Ser	Gln
				340					345					350		

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(continued)

	His	Leu	Leu	Leu	Asp	Asn	Lys	Gly	His	Leu	Phe	Asp	Ile	Lys	Ser	Thr	
		365						360					365				
5	Ala	Thr	Ser	Tyr	Ser	Val	Leu	His	Asn	Ser	His	Pro	Gly	Glu	Ile	Lys	
		370					375					380					
	Gly	Lys	Leu	Ala	Gln	Ala	Gly	Thr	Gly	Ser	Val	Ser	Val	Asp	Gly	Lys	
10		385				390					395					400	
	Ser	Gly	Lys	Ile	Ser	Leu	Gly	Ser	Gly	Thr	Gln	Ser	His	Asn	Lys	Thr	
				405						410					415		
	Met	Leu	Ser	Gln	Pro	Gly	Glu	Ala	His	Arg	Ser	Leu	Leu	Thr	Gly	Ile	
15				420					425					430			
	Trp	Gln	His	Pro	Ala	Gly	Ala	Ala	Arg	Pro	Gln	Gly	Glu	Ser	Ile	Arg	
			435				440						445				
20	Leu	His	Asp	Asp	Lys	Ile	His	Ile	Leu	His	Pro	Glu	Leu	Gly	Val	Trp	
		450					455					460					
	Gln	Ser	Ala	Asp	Lys	Asp	Thr	His	Ser	Gln	Leu	Ser	Arg	Gln	Ala	Asp	
25		465				470					475					480	
	Gly	Lys	Leu	Tyr	Ala	Leu	Lys	Asp	Asn	Arg	Thr	Leu	Gln	Asn	Leu	Ser	
				485						490					495		
30	Asp	Asn	Lys	Ser	Ser	Glu	Lys	Leu	Val	Asp	Lys	Ile	Lys	Ser	Tyr	Ser	
			500						505					510			
	Val	Asp	Gln	Arg	Gly	Gln	Val	Ala	Ile	Leu	Thr	Asp	Thr	Pro	Gly	Arg	
		515						520					525				
35	His	Lys	Met	Ser	Ile	Met	Pro	Ser	Leu	Asp	Ala	Ser	Pro	Glu	Ser	His	
		530					535					540					
	Ile	Ser	Leu	Ser	Leu	His	Phe	Ala	Asp	Ala	His	Gln	Gly	Leu	Leu	His	
40		545				550					555					560	
	Gly	Lys	Ser	Glu	Leu	Glu	Ala	Gln	Ser	Val	Ala	Ile	Ser	His	Gly	Arg	
				565						570					575		
45	Leu	Val	Val	Ala	Asp	Ser	Glu	Gly	Lys	Leu	Phe	Ser	Ala	Ala	Ile	Pro	
			580						585					590			
	Lys	Gln	Gly	Asp	Gly	Asn	Glu	Leu	Lys	Met	Lys	Ala	Met	Pro	Gln	His	
		595						600					605				
50	Ala	Leu	Asp	Glu	His	Phe	Gly	His	Asp	His	Gln	Ile	Ser	Gly	Phe	Phe	
		610					615					620					
	His	Asp	Asp	His	Gly	Gln	Leu	Asn	Ala	Leu	Val	Lys	Asn	Asn	Phe	Arg	
55		625				630					635					640	
	Gln	Gln	His	Ala	Cys	Pro	Leu	Gly	Asn	Asp	His	Gln	Phe	His	Pro	Gly	
				645						650					655		
60	Trp	Asn	Leu	Thr	Asp	Ala	Leu	Val	Ile	Asp	Asn	Gln	Leu	Gly	Leu	His	
			660						665					670			
	His	Thr	Asn	Pro	Glu	Pro	His	Glu	Ile	Leu	Asp	Met	Gly	His	Leu	Gly	
		675						680					685				

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Ser	Leu	Ala	Leu	Gln	Glu	Gly	Lys	Leu	His	Tyr	Phe	Asp	Gln	Leu	Thr
690						695					700				
Lys	Gly	Trp	Thr	Gly	Ala	Glu	Ser	Asp	Cys	Lys	Gln	Leu	Lys	Lys	Gly
705					710					715					720
Leu	Asp	Gly	Ala	Ala	Tyr	Leu	Leu	Lys	Asp	Gly	Glu	Val	Lys	Arg	Leu
				725					730					735	
Asn	Ile	Asn	Gln	Ser	Thr	Ser	Ser	Ile	Lys	His	Gly	Thr	Glu	Asn	Val
			740					745					750		
Phe	Ser	Leu	Pro	His	Val	Arg	Asn	Lys	Pro	Glu	Pro	Gly	Asp	Ala	Leu
		755					760					765			
Gln	Gly	Leu	Asn	Lys	Asp	Asp	Lys	Ala	Gln	Ala	Met	Ala	Val	Ile	Gly
						775					780				
Val	Asn	Lys	Tyr	Leu	Ala	Leu	Thr	Glu	Lys	Gly	Asp	Ile	Arg	Ser	Phe
785					790					795					800
Gln	Ile	Lys	Pro	Gly	Thr	Gln	Gln	Leu	Glu	Arg	Pro	Ala	Gln	Thr	Leu
				805					810					815	
Ser	Arg	Glu	Gly	Ile	Ser	Gly	Glu	Leu	Lys	Asp	Ile	His	Val	Asp	His
			820					825					830		
Lys	Gln	Asn	Leu	Tyr	Ala	Leu	Thr	His	Glu	Gly	Glu	Val	Phe	His	Gln
		835					840					845			
Pro	Arg	Glu	Ala	Trp	Gln	Asn	Gly	Ala	Glu	Ser	Ser	Ser	Trp	His	Lys
						855					860				
Leu	Ala	Leu	Pro	Gln	Ser	Glu	Ser	Lys	Leu	Lys	Ser	Leu	Asp	Met	Ser
865					870					875					880
His	Glu	His	Lys	Pro	Ile	Ala	Thr	Phe	Glu	Asp	Gly	Ser	Gln	His	Gln
				885					890					895	
Leu	Lys	Ala	Gly	Gly	Trp	His	Ala	Tyr	Ala	Ala	Pro	Glu	Arg	Gly	Pro
			900					905					910		
Leu	Ala	Val	Gly	Thr	Ser	Gly	Ser	Gln	Thr	Val	Phe	Asn	Arg	Leu	Met
		915					920					925			
Gln	Gly	Val	Lys	Gly	Lys	Val	Ile	Pro	Gly	Ser	Gly	Leu	Thr	Val	Lys
						935					940				
Leu	Ser	Ala	Gln	Thr	Gly	Gly	Met	Thr	Gly	Ala	Glu	Gly	Arg	Lys	Val
945					950					955					960
Ser	Ser	Lys	Phe	Ser	Glu	Arg	Ile	Arg	Ala	Tyr	Ala	Phe	Asn	Pro	Thr
				965					970					975	
Met	Ser	Thr	Pro	Arg	Pro	Ile	Lys	Asn	Ala	Ala	Tyr	Ala	Thr	Gln	His
			980					985					990		
Gly	Trp	Gln	Gly	Arg	Glu	Gly	Leu	Lys	Pro	Leu	Tyr	Glu	Met	Gln	Gly
		995					1000					1005			
Ala	Leu	Ile	Lys	Gln	Leu	Asp	Ala	His	Asn	Val	Arg	His	Asn	Ala	Pro
						1015					1020				
Gln	Pro	Asp	Leu	Gln	Ser	Lys	Leu	Glu	Thr	Leu	Asp	Leu	Gly	Glu	His
1025					1030					1035					1040

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Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu
1045 1050 1055

Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val
1060 1065 1070

Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly
1075 1080 1085

Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu
1090 1095 1100

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Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu
1105 1110 1115 1120

Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp
1125 1130 1135

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Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro
1140 1145 1150

Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val
1155 1160 1165

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Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser
1170 1175 1180

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Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe
1185 1190 1195 1200

Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr
1205 1210 1215

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Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp
1220 1225 1230

Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val
1235 1240 1245

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Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu
1250 1255 1260

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Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser
1265 1270 1275 1280

Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val
1285 1290 1295

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Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly
1300 1305 1310

Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly
1315 1320 1325

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Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile
1330 1335 1340

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Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys
1345 1350 1355 1360

Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile
1365 1370 1375

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Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val
1730 1735 1740
Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser
1745 1750 1755 1760
Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu
1765 1770 1775
Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile
1780 1785 1790
Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg
1795 1800 1805
Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser
1810 1815 1820
Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser
1825 1830 1835

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

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The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 29 as follows:

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ATGACATCGT CACAGCAGCG GGTGAAAGG TTTTACAGT ATTTCTCCGC CGGGTGTAAG 60
ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAGA TGAGGAGGCG 120
GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG 180
GCTGACCCAC AAACCTCAAT AACCTGTAT TCGATGCTAT TACAGCTGAA TTTTGAAATG 240
GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACTGC ACAACGTGCG TTTATGTTTT 300
CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTTCGCG ATATCGTTAG CGGCTTCATC 360
GAACATGCGG CAGAAGTGCG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA 420

This is known as the dspF gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 30 as follows:

50

55

Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser
1 5 10 15
Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu
20 25 30
Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His
35 40 45
Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln
50 55 60

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Sub
AS

Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
 65 70 75 80
 Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val
 85 90 95
 Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe
 100 105 110
 Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu
 115 120 125
 Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala
 130 135

This protein or polypeptide is about 16 kDa and has a pI of 4.45.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID.

No. 31 as follows:

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
 1 5 10 15
 Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
 20 25 30
 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
 35 40 45
 Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
 50 55 60
 Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
 65 70 75 80
 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
 85 90 95
 Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
 100 105 110
 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
 115 120 125
 Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
 130 135 140
 Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
 145 150 155 160
 Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
 165 170 175
 Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
 180 185 190

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87

5 Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
195 200 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
210 215 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225 230 235 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
245 250 255

10 Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
260 265 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
275 280 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
290 295 300

15 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305 310 315 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
325 330 335

20 Asn Gln Ala Ala Ala
340

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.

25 Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer, "Pseudomonas syringae pv. syringae Harpin_{PSS}: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the

30 hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 32 as follows:

ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCA^{~1}AACCC CGGCAATGGC CCTGTGCCTG 60

GTACGTCTCG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC 120

35 GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTC^{~1}AACTCG ACGACAGCTC GCCATTGGGA 180

AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC 240

ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG 300

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GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC 360
AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC 420
GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC 480
AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC 540
GAAACGGCTG CGTTCCGTTC GGCACCTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG 600
AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC 660
AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC 720
GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA 780
TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCAGAGAC CGGTACGTCG 840
GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG 900
GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT 960
GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA 1020
GCCTGA 1026

15

Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817, which is hereby incorporated by reference. The protein has a nucleotide sequence of SEQ. ID. No. 33 as follows:

20

25

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TCCACTTCGC TGATTTTGAA ATTGGCAGAT TCATAGAAAC GTTCAGGTGT GGAAATCAGG 60
CTGAGTGC GC AGATTTCTGTT GATAAGGCTG TGGTACTGGT CATTGTTGGT CATTTCAGG 120
CCTCTGAGTG CGGTGCGGAG CAATACCACT CTTCCTGCTG GCGTGTGCAC ACTGAGTCGC 180
AGGCATAGGC ATTTCACTTC CTTCGCTTGG TTGGGCATAT AAAAAAAGGA ACTTTTAAAA 240
ACAGTGCAAT GAGATGCCGG CAAAACGGGA ACCGGTCGCT GCGCTTTGCC ACTCACTTCG 300
AGCAAGCTCA ACCCCAAACA TCCACATCCC TATCGAACGG ACAGCGATAC GGCCACTTGC 360
TCTGGTAAAC CCTGGAGCTG GCGTCGGTCC AATTGCCAC TTAGCGAGGT AACGCAGCAT 420
GAGCATCGGC ATCACACCCC GGCCGCAACA GACCAACAG CCACTCGATT TTTCGGCGCT 480
AAGCGGCAAG AGTCCTCAAC CAAACACGTT CGGCGAGCAG AACACTCAGC AAGCGATCGA 540
CCCGAGTGCA CTGTTGTTCG GCAGCGACAC ACAGAAAGAC GTCAACTTCG GCACGCCCGA 600
CAGCACCCTC CAGAATCCGC AGGACGCCAG CAAGCCCAAC GACAGCCAGT CCAACATCGC 660
TAAATTGATC AGTGCAATTGA TCATGTCGTT GCTGCAGATG CTCACCAACT CCAATAAAAA 720
GCAGGACACC AATCAGGAAC AGCCTGATAG CCAGGCTCCT TTCCAGAAACA ACGGCGGGCT 780

CGGTACACCG TCGGCCGATA GCGGGGGCGG CGGTACACCG GATGCGACAG GTGGCGGCGG 840
 5 CGGTGATACG CCAAGCGCAA CAGGCGGTGG CGGCGGTGAT ACTCCGACCG CAACAGGCGG 900
 TGGCGGCAGC GGTGGCGGCG GCACACCCAC TGCAACAGGT GGCGGCAGCG GTGGCACACC 960
 CACTGCAACA GGCGGTGGCG AGGGTGGCGT AACACCGCAA ATCACTCCGC AGTTGGCCAA 1020
 10 CCCTAACCGT ACCTCAGGTA CTGGCTCGGT GTCGGACACC GCAGGTTCTA CCGAGCAAGC 1080
 CGGCAAGATC AATGTGGTGA AAGACACCAT CAAGGTCGGC GCTGGCGAAG TCTTTGACGG 1140
 CCACGGCGCA ACCTTCACTG CCGACAAATC TATGGGTAAC GGAGACCAGG GCGAAAATCA 1200
 15 GAAGCCCATG TTCGAGCTGG CTGAAGGCGC TACGTTGAAG AATGTGAACC TGGGTGAGAA 1260
 CGAGGTCGAT GGCATCCACG TGAAAGCCAA AAACGCTCAG GAAGTCACCA TTGACAACGT 1320
 20 GCATGCCCAG AACGTCGGTG AAGACCTCAT TACGGTCAAA GGCGAGGGAG GCGCAGCGGT 1380
 CACTAATCTG AACATCAAGA ACAGCAGTGC CAAAGGTGCA GACGACAAGG TTGTCCAGCT 1440
 CAACGCCAAC ACTCACTTGA AAATCGACAA CTTCAAGGCC GACGATTTTCG GCACGATGGT 1500
 25 TCGCACCAAC GGTGGCAAGC AGTTTGATGA CATGAGCATC GAGCTGAACG GCATCGAAGC 1560
 TAACCACGGC AAGTTCGCCC TGGTGAAAAG CGACAGTGAC GATCTGAAGC TGGCAACGGG 1620
 30 CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA 1680
 CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAGGG GGTGGACTC 1729

35 This DNA molecule is known as the dspE gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 34 as follows:

40 Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
 1 5 10 15
 Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
 20 25 30
 45 Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
 35 40 45
 Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
 50 55 60
 Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
 65 70 75 80
 55 Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
 85 90 95

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Asn	Ser	Asn	Lys	Lys	Gln	Asp	Thr	Asn	Gln	Glu	Gln	Pro	Asp	Ser	Gln
			100					105					110		
Ala	Pro	Phe	Gln	Asn	Asn	Gly	Gly	Leu	Gly	Thr	Pro	Ser	Ala	Asp	Ser
		115					120					125			
Gly	Gly	Gly	Gly	Thr	Pro	Asp	Ala	Thr	Gly	Gly	Gly	Gly	Gly	Asp	Thr
	130					135					140				
Pro	Ser	Ala	Thr	Gly	Gly	Gly	Gly	Gly	Asp	Thr	Pro	Thr	Ala	Thr	Gly
145				150						155					160
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Thr	Pro	Thr	Ala	Thr	Gly	Gly	Gly
				165					170					175	
Ser	Gly	Gly	Thr	Pro	Thr	Ala	Thr	Gly	Gly	Gly	Glu	Gly	Gly	Val	Thr
			180					185					190		
Pro	Gln	Ile	Thr	Pro	Gln	Leu	Ala	Asn	Pro	Asn	Arg	Thr	Ser	Gly	Thr
		195					200					205			
Gly	Ser	Val	Ser	Asp	Thr	Ala	Gly	Ser	Thr	Glu	Gln	Ala	Gly	Lys	Ile
	210					215					220				
Asn	Val	Val	Lys	Asp	Thr	Ile	Lys	Val	Gly	Ala	Gly	Glu	Val	Phe	Asp
225					230					235					240
Gly	His	Gly	Ala	Thr	Phe	Thr	Ala	Asp	Lys	Ser	Met	Gly	Asn	Gly	Asp
				245					250					255	
Gln	Gly	Glu	Asn	Gln	Lys	Pro	Met	Phe	Glu	Leu	Ala	Glu	Gly	Ala	Thr
			260					265					270		
Leu	Lys	Asn	Val	Asn	Leu	Gly	Glu	Asn	Glu	Val	Asp	Gly	Ile	His	Val
		275					280					285			
Lys	Ala	Lys	Asn	Ala	Gln	Glu	Val	Thr	Ile	Asp	Asn	Val	His	Ala	Gln
	290					295					300				
Asn	Val	Gly	Glu	Asp	Leu	Ile	Thr	Val	Lys	Gly	Glu	Gly	Gly	Ala	Ala
305					310					315					320
Val	Thr	Asn	Leu	Asn	Ile	Lys	Asn	Ser	Ser	Ala	Lys	Gly	Ala	Asp	Asp
				325					330					335	
Lys	Val	Val	Gln	Leu	Asn	Ala	Asn	Thr	His	Leu	Lys	Ile	Asp	Asn	Phe
			340					345					350		
Lys	Ala	Asp	Asp	Phe	Gly	Thr	Met	Val	Arg	Thr	Asn	Gly	Gly	Lys	Gln
		355					360					365			
Phe	Asp	Asp	Met	Ser	Ile	Glu	Leu	Asn	Gly	Ile	Glu	Ala	Asn	His	Gly
	370					375					380				
Lys	Phe	Ala	Leu	Val	Lys	Ser	Asp	Ser	Asp	Asp	Leu	Lys	Leu	Ala	Thr
385					390					395					400
Gly	Asn	Ile	Ala	Met	Thr	Asp	Val	Lys	His	Ala	Tyr	Asp	Lys	Thr	Gln
				405					410					415	

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Ala Ser Thr Gln His Thr Glu Leu
420

5

This protein or polypeptide is about 42.9 kDa.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ.

10 ID. No. 35 as follows:

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1 5 10 15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
20 25 30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
35 40 45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
50 55 60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65 70 75 80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
85 90 95

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
100 105 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
115 120 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
130 135 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
145 150 155 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
165 170 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
180 185 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
195 200 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
210 215 220

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SEQ. ID. NO. 35

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5 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
225 230 235 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
245 250 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln
260 265 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
275 280 285

10 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
290 295 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
305 310 315 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
325 330 335

15 Gln Ser Thr Ser Thr Gln Pro Met
340

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ.
ID. No. 36 as follows:

20 ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC 60

AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC 120

GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCA CA GTCGGCGGGC 180

GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC 240

AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC 300

GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATCA GCTGCTGGAA 360

25 GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG 420

GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC 480

GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC 540

GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT 600

GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC 660

30 GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC 720

CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG 780

ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC 840

GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT 900

GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC 960
GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG 1020
ACGCAGCCGA TGTAA 1035

5 Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted
10 via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 37 as follows:

15 Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
1 5 10 15
Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
20 25

20 This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other
25 *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 38 as follows:

30 Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1 5 10 15
Leu Leu Ala Met
20

35 Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*

subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves,” MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., “Harpin is Not
5 Necessary for the Pathogenicity of *Erwinia stewartii* on Maize,” 8th Int’l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., “Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize,” Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from
10 *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamoni*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., “Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens,” Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., “Structure and
15 Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco,” Eur. J. Biochem., 183:555-63 (1989), Ricci et al., “Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*,” Plant Path. 41:298-307 (1992), Baillreul et al., “A New Elicitor of the Hypersensitive Response in Tobacco: A
20 Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance,” Plant J., 8(4):551-60 (1995), and Bonnet et al., “Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants,” Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

25 Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

The above elicitors are exemplary. Other elicitors can be identified by
30 growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture

supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do not elicit a hypersensitive response include fragments of the *Erwinia amylovora* hypersensitive response elicitor. Suitable fragments include a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, or an internal fragment of the amino acid sequence of SEQ. ID. No. 23. The C-terminal fragment of the amino acid

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sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403. The internal fragment of the amino acid sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156. Other suitable fragments can be identified in accordance with the present invention.

Another example of a useful fragment of a hypersensitive response elicitor which fragment does not itself elicit a hypersensitive response is the protein fragment containing amino acids 190 to 294 of the amino acid sequence (SEQ. ID. No. 31) for the *Pseudomonas syringae* pv. *syringae* hypersensitive response elicitor. This fragment is useful in imparting disease resistance and enhancing plant growth.

Yet another example of a useful fragment of a hypersensitive response elicitor is the peptide having an amino acid sequence corresponding to SEQ. ID. No. 39. This peptide is derived from the hypersensitive response eliciting glycoprotein of *Phytophthora megasperma* and enhances plant growth.

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The fragment of the present invention is preferably in isolated form (i.e. separated from its host organism) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the fragment of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein fragment, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the fragment is separated by centrifugation. The supernatant fraction containing the fragment is subjected to gel filtration in an

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appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using
5 conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the
10 inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA
15 ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

20 Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which
25 is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or
30 electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A

Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

5 A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression
10 elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

15 Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promoters differ from those of procaryotic promoters. Furthermore, eucaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a
20 procaryotic system, and, further, procaryotic promoters are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called
25 the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct
30 positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the fragment of a hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying the fragment of a hypersensitive response elicitor polypeptide or protein which does not elicit a hypersensitive response in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the fragment to impart disease resistance, enhance growth, and/or control insects. Alternatively, these fragments of a hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, and/or to effect insect control.

As an alternative to applying a fragment of a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, and/or to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated fragment or 2) application of bacteria which do not cause disease and are transformed with a gene encoding the fragment. In the latter embodiment, the fragment can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response. Such bacteria must be capable of secreting or exporting the fragment so that the fragment can contact plant or plant seed cells. In these embodiments, the fragment is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of

pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention:

- 5 *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

With regard to the use of the fragments of the hypersensitive response
10 elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds
15 germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed
20 germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

Another aspect of the present invention is directed to effecting any
25 form of insect control for plants. For example, insect control according to the present invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from
30 releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding
5 pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect
10 pests represents the most economically important group of pests for vegetable production worldwide.

The method of the present invention involving application of the fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots,
15 propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the fragment of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant
20 seeds or propagules (e.g., cuttings), in accordance with the application embodiment of the present invention, the fragment of the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application
25 procedures can be envisioned by those skilled in the art provided they are able to effect contact of the fragment with cells of the plant or plant seed. Once treated with the fragment of the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in
30 accordance with the present invention, the plants may be treated with one or more applications of the fragment of the hypersensitive response elicitor protein or polypeptide or whole elicitors to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

The fragment of the hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the fragment can be applied separately to plants with other materials being applied at different times.

5 A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains
10 greater than 500 nM of the fragment.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include $(\text{NH}_4)_2\text{NO}_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

15 Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response eliciting fragment can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

20 In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a fragment of a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such a fragment are produced according to procedures well known in the art.

25 The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby
30 incorporated by reference.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic

transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy

root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves
5 can be cultivated in accordance with conventional procedure with the presence of the gene encoding the fragment of the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant.

Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using
10 conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the
15 polypeptide or protein fragment.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a fragment of a hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including
20 a fragment of a hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the fragment of a
25 hypersensitive response elicitor in accordance with the present invention to impart disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

EXAMPLES

30 Example 1 - Bacterial Strains and Plasmids

Escherichia coli strains used in the following examples include DH5 α and BL21(DE3) purchased from Gibco BRL (Grand Island, N.Y.) and Stratagene

(La Jolla, CA), respectively. The pET28(b) vector was purchased from Novagen (Madison, WI). Eco DH5 α /2139 contained the complete *hrpN* gene. The 2139 construct was produced by D. Bauer at Cornell University. The *hrpN* gene was cleaved from the 2139 plasmid by restriction enzyme digestion with HindIII, then purified from an agarose gel to serve as the DNA template for PCR synthesis of truncated *hrpN* clones. These clones were subsequently inserted into the (His)₆ vector pET28(b) which contained a Kan^r gene for selection of transformants.

Example 2 - DNA Manipulation

Restriction enzymes were obtained from Boehringer Mannheim (Indianapolis, IN) or Gibco BRL. T4 DNA ligase, Calf Intestinal Alkaline Phosphatase (CIAP), and PCR SupermixTM were obtained from Gibco BRL. The QIAprep Spin Miniprep Kit, the Qiagen Plasmid Mini Kit, and the QIAquick PCR Purification Kit were purchased from Qiagen (Hilden, Germany). The PCR primers were synthesized by Lofstrand Labs Limited (Gaithersburg, MD). The oligopeptides were synthesized by Bio-Synthesis, Inc. (Lewisville, TX). All DNA manipulations such as plasmid isolation, restriction enzyme digestion, DNA ligation, and PCR were performed according to standard techniques (Sambrook, et al., Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press (1989)) or protocols provided by the manufacturer.

Example 3 - Fragmentation of *hrpN* Gene

A series of N-terminal and C-terminal truncated *hrpN* genes and internal fragments were generated via PCR (Fig. 1). The full length *hrpN* gene was used as the DNA template and 3' and 5' primers were designed for each truncated clone (Fig. 2). The 3' primers contained an NdeI enzyme cutting site which contained the start codon ATG (methionine) and the 5' primers contained the stop codon TAA and a HindIII enzyme cutting site for ligation into the pET28(b) vector. PCR was carried out in 0.5 ml tubes in a GeneAmpTM 9700 (Perkin-Elmer, Foster City, CA). 45 μ l of SupermixTM (Life Technology, Gaithersburg, MD) were mixed with 20 pmoles of each pair of DNA primers, 10 ng of full length harpin DNA, and deionized

H₂O to a final volume of 50 µl. After heating the mixture at 95°C for 2 min, the PCR was performed for 30 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1.5 min. The PCR products were verified on a 6% TBE gel (Novex, San Diego, CA). Amplified DNA was purified with the QIAquick PCR purification kit, digested with Nde I and Hind III at 37°C for 5 hours, extracted once with phenol:chloroform:isoamylalcohol (25:25:1) and precipitated with ethanol. 5 µg of pET28(b) vector DNA were digested with 15 units of Nde I and 20 units of Hind III at 37°C for 3 hours followed with CIAP treatment to reduce the background resulting from incomplete single enzyme digestion. Digested vector DNA was purified with the QIAquick PCR purification kit and directly used for ligation. Ligation was carried out at 14-16°C for 5-12 hours in a 15 µl mixture containing ca. 200 ng of digested pET28(b), 30 ng of targeted PCR fragment, and 1 unit T4 DNA ligase. 5 - 7.5 µl of ligation solution were added to 100 µl of DH5α competent cells in a 15 ml Falcon tube and incubated on ice for 30 min. After a heat shock at 42°C for 45 seconds, 0.9 ml SOC solution or 0.45 ml LB media were added to each tube and incubated at 37°C for 1 hour. 20, 100, and 200 µl of transformed cells were placed onto LB agar with 30 µg/ml of kanamycin and incubated at 37°C overnight. Single colonies were transferred to 3 ml LB-media and incubated overnight at 37°C. Plasmid DNA was prepared from 2 ml of culture with the QIAprep Miniprep kit (QIAGEN, Hilden, Germany). The DNA from the transformed cells was analyzed by restriction enzyme digestion or partial sequencing to verify the success of the transformations. Plasmids with the desired DNA sequence were transferred into the BL21 strain using the standard chemical transformation method as indicated above. A clone containing the full length harpin protein in the pET28(b) vector was generated as a positive control, and a clone with only the pET28(b) vector was generated as a negative control.

Example 4 - Expression of Hypersensitive Response Elicitor Truncated Proteins

Escherichia coli BL21(DE3) strains containing the hrpN clones were grown in Luria broth medium (5g/L Difco Yeast extract, 10 g/L Difco Tryptone, 5 g/L NaCl, and 1 mM NaOH) containing 30 µg/ml of kanamycin at 37°C overnight. The bacteria were then inoculated into 100 volumes of the same medium and grown at

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37°C to an OD₆₂₀ of 0.6-0.8. The bacteria were then inoculated into 250 volumes of the same medium and grown at 37°C to an OD₆₂₀ of ca. 0.3 or 0.6-0.8. One millimolar IPTG was then added and the cultures grown at 19°C overnight (ca. 18 hours). Not all of the clones were successfully expressed using this strategy. Several of the clones had to be grown in Terrific broth (12 g/L Bacto Tryptone, 24 g/L Bacto yeast, 0.4% glycerol, 0.17 M KH₂PO₄, and 0.72 K₂HPO₄), and/or grown at 37°C after IPTG induction, and/or harvested earlier than overnight (Table 1).

Table 1: Expression of hypersensitive response elicitor truncated proteins

Fragment	amino acids (SEQ. ID. No. 23)	Growth medium	Induction O.D.	Expression temp.	Harvest time
1 (+ control)	1-403	LB	ca. 0.3 or 0.6-0.8	19°C or 25°C	16-18 hr
2 (+ control)	-	LB and TB	ca. 0.3 or 0.6-0.8	19 C and 37 C	16-18 hr
3	105-403	LB	0.6-0.8	19°C	16-18 hr
4	169-403	TB	ca. 0.3	19°C	16-18 hr
5	210-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
6	257-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
7	343-403	LB	ca. 0.3	19°C	5 hr
8	1-75	TB	ca. 0.3	37°C	16-18 hr
9	1-104	TB	ca. 0.3	37°C	16-18 hr
10	1-168	TB	ca. 0.3	37°C	16-18 hr
11	1-266	LB	ca. 0.3	37°C	4 hr
12	1-342	LB	0.6-0.8	19°C	16-18 hr
13	76-209	LB	ca. 0.3	37°C	5 hr
14	76-168	TB or LB	ca. 0.3	37°C	3 hr or 16-18 hr
15	105-209	M9ZB	ca. 0.3	37°C	3 hr
16	169-209	no expression			
17	105-168	LB	ca. 0.3	37°C	3-5 hr
18	99-209	LB	ca. 0.3	37°C	3 hr
19	137-204	LB	ca. 0.3	37°C	3 hr
20	137-180	LB	ca. 0.3	37°C	16-18 hr.
21	105-180	LB	ca. 0.3	37°C	3 hr
22	150-209	no expression			
23	150-180	no expression			

Example 5 - Small Scale Purification of Hypersensitive Response Elicitor Truncated Proteins (Verification of Expression)

A 50 ml culture of a hrpN clone was grown as above to induce expression of the truncated protein. Upon harvesting of the culture, 1.5 ml of the cell

suspension were centrifuged at 14,000 rpm for 5 minutes, re-suspended in urea lysis buffer (8 M urea, 0.1 M Na₂HPO₄, and 0.01 M Tris -- pH 8.0), incubated at room temperature for 10 minutes, then centrifuged again at 14,000 rpm for 10 minutes, and the supernatant saved. A 50 µl aliquot of a 50% slurry of an equilibrated (His)₆-
5 binding nickel agarose resin was added to the supernatant and mixed at 4°C for one hour. The nickel agarose was then washed three times with urea washing buffer (8 M urea, 0.1 M Na₂HPO₄, and 0.01 M Tris -- pH 6.3), centrifuging at 5,000 rpm for five minutes between washings. The protein was eluted from the resin with 50 µl of urea elution buffer (8 M urea, 0.1 M Na₂HPO₄, 0.01 M Tris, and 0.1 M EDTA -- pH 6.3).
10 The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression.

Example 6 - Induction of HR in Tobacco

A 1.5 ml aliquot from the 50 ml cultures grown for small scale
15 purification of the truncated proteins was centrifuged at 14,000 rpm for four minutes and re-suspended in an equal volume of 5 mM potassium phosphate buffer, pH 6.8. The cell suspension was sonicated for ca. 30 seconds then diluted 1:2 and 1:10 with phosphate buffer. Both dilutions plus the neat cell lysate were infiltrated into the fourth to ninth leaves of 10-15 leaf tobacco plants by making a hole in single leaf
20 panes and infiltrating the bacterial lysate into the intercellular leaf space using a syringe without a needle. The HR response was recorded 24-48 hr post infiltration. Tobacco (*Nicotiana tabacum* v. Xanthi) seedlings were grown in an environmental chamber at 20-25°C with a photoperiod of 12-h light /12-h dark and ca. 40% RH. Cell lysate was used for the initial HR assays (in order to screen the truncated proteins
25 for HR activity) as the small scale urea purification yielded very little protein which was denatured due to the purification process.

Example 7 - Large Scale Native Purification of Hypersensitive Response Elicitor Truncated Proteins for Comprehensive Biological Activity Assays

30 Six 500 ml cultures of a hrpN clone were grown as described earlier to induce expression of the truncated protein. Upon harvesting of the culture, the cells were centrifuged at 7,000 rpm for 5 minutes, re-suspended in imidazole lysis buffer (5

mM imidazole, 0.5 M NaCl, 20 mM Tris) plus Triton X-100 at 0.05% and lysozyme at 0.1 mg/ml, incubated at 30°C for 15 minutes, sonicated for two minutes, centrifuged again at 15,000 rpm for 20 minutes, and the supernatant was saved. A 4 ml aliquot of a 50% slurry of an equilibrated (His)₆-binding nickel agarose resin was added to the supernatant and mixed at 4°C for ca. four hours. The nickel agarose was then washed three times with imidazole washing buffer (20 mM imidazole, 0.5 M NaCl, and 20 mM Tris), centrifuging at 5,000 rpm for five minutes between washings, then placed in a disposable chromatography column. The column was centrifuged at 1100 rpm for one minute to remove any residual wash buffer and then the protein was eluted from the resin with 4 ml of imidazole elution buffer (1 M imidazole, 0.5 M NaCl, and 20 mM Tris) by incubating the column with the elution buffer for ten minutes at room temperature and then centrifuging the column at 1100 rpm for one minute. The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression. The concentration of the proteins was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker.

Example 8 - Large Scale Urea Purification of Hypersensitive Response Elicitor Truncated Proteins For Comprehensive Biological Activity Assay

The procedure was the same as the large scale native purification except that urea lysis buffer, washing buffer, and elution buffer were used, and the cells were not sonicated as in the native purification. After purification, the protein was renatured by dialyzing against lower and lower concentrations of urea over an eight hour period, then dialyzing overnight against 10 mM Tris/20 mM NaCl. The renaturing process caused the N-terminal proteins to precipitate. The precipitated 1-168 protein was solubilized by the addition of 100 mM Tris-HCl at pH 10.4 then heating the protein at 30°C for ca. one hour. The concentration of the protein was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker. The 1-75 and 1-104 protein fragments were not successfully solubilized using this strategy so they were sonicated in 100 mM Tris-HCl at pH 10.4 to solubilize as much of the protein as possible and expose the active sites of the protein for the biological activity assays.

Example 9 – Induction of Growth Enhancement (GE)

Sixty tomato (*Lycopersicon spp.* cv. Marglobe) seeds were soaked
5 overnight in 10 and 20 µg/ml of the truncated protein diluted with 5mM potassium
phosphate buffer, pH 6.8. The next morning, the sixty seeds were sewn in three pots
and 12-15 days later and again 18-20 days later the heights of the 10 tallest tomato
plants per pot were measured and compared with the heights of the control plants
treated only with phosphate buffer. Analyses were done on the heights to determine if
10 there was a significant difference in the height of the plants treated with the truncated
proteins compared with the buffer control, and thereby determine whether the proteins
induced growth enhancement.

Example 10 – Induction of Systemic Acquired Resistance (SAR)

15 Three tobacco (*Nicotiana tabacum* cv. Xanthi) plants with 8-12 leaves
(ca. 75 day old plants) were used in the assay. One leaf of the tobacco plants was
covered up and the rest of the leaves were sprayed with ca. 50 ml of a 20 µg/ml
solution of the truncated proteins diluted with 5mM potassium phosphate buffer. Five
20 to seven days later two leaves (the unsprayed leaf and the sprayed leaf opposite and
just above the unsprayed leaf) were inoculated with 20 µl of a 1.8 µg/ml solution of
TMV along with a pinch of diatomaceous earth by rubbing the mixture along the top
surface of the leaves. The TMV entered the plants through tiny lesions made by the
diatomaceous earth. Ca. 3-4 days post TMV inoculation, the number of TMV lesions
25 was counted on both leaves compared with the number of lesions on the negative
control buffer treated leaves. Analyses were done to determine the efficacy of
reducing the number of TMV lesions by the protein fragments compared to the buffer
control. Percentage of efficacy was calculated as: Reduction in TMV lesions (%
efficacy) = 100 x (1 – mean # of lesions on treated leaves/mean # of lesions on buffer
30 control leaves).

Example 11 - Expression of Hypersensitive Response Elicitor Truncated Proteins

The small scale expression and purification of the fragment proteins was done to screen for expression and HR activity (Table 2).

Table 2

Expression and HR activity of hypersensitive response elicitor truncated proteins (small scale screening)

Fragment #	Amino Acids (SEQ. ID. No. 23)	Expression	HR activity
1(+control)	1-403	+	+
2(- control)	-	background protein only	-
3	105-403	+	+
4	169-403	+	-
5	210-403	+	-
6	267-403	+	-
7	343-403	+/-	-
8	1-75	+	-
9	1-104	+	+/-
10	1-168	+	+
11	1-266	+	+
12	1-342	+	+
13	76-209	+	+
14	76-168	+	-
15	105-209	+	+
16	169-209	-	-
17	105-168	+	-
18	99-209	+	± ^a
19	137-204	+	+
20	137-180	+	+
21	105-180	+	+
22	150-209	-	-
23	150-180	-	-

All of the cloned fragment proteins were expressed at varying levels except for three small fragments (amino acids 169-209, 150-209, and 150-180). Fragments 210-403 and 267-403 were expressed very well, yielding a high concentration of protein from a small scale purification, resulting in a substantial protein band on SDS gel electrophoresis. Other fragments (such as a.a. 1-168 and 1-104) produced much less protein, resulting in faint protein bands upon electrophoresis. It was difficult to determine whether fragment 343-403, the smallest C-terminal protein, was expressed, as there were several background proteins apparent on the gel, in addition to the suspected 343-403 protein. The positive and negative control proteins, consisting of

the full length hypersensitive response elicitor protein and only background proteins, respectively, were tested for expression and HR activity as well.

The large scale expression and purification of the fragment proteins was done to determine the level of expression and titer of the HR activity (Table 3).

Table 3

Expression level and HR titer of hypersensitive response elicitor truncated proteins (large scale purification)

Fragment #	Amino acids (SEQ. ID. No. 23)	Expression	HR titer
1 (+ control)	1-403	3.7 mg/ml	5-7 µg/ml
2 (- control)	-	-	1:2 dilution
4	169-403	2 mg/ml	-
5	210-403	5 mg/ml	-
6	267-403	4 mg/ml	-
7	343-402	200 µg/ml	-
8	1-75	50 µg/ml	-
9	1-104	50 µg/ml	3 µg/ml (1:16 dilution)
10	1-168	1 mg/ml	1 µg/ml
13	76-209	2.5 mg/ml	5 µg/ml
14	76-168	2 mg/ml	-
15	105-209	5 mg/ml	5-10 µg/ml
17	105-168	250 µg/ml	-
19	137-204	3.6 mg/ml	3.5 µg/ml
20	137-180	250 µg/ml	16 µg/ml

The truncated proteins deemed to be the most important in characterizing the hypersensitive response elicitor were chosen for large scale expression. The positive control (full length hypersensitive response elicitor) was expressed at a relatively high level at 3.7 mg/ml. All of the C-terminal proteins were expressed at relatively high levels from 2-5 mg/ml, except for fragment 343-403 as discussed earlier. The N-terminal fragments were expressed very well also; however, during the purification process, the protein precipitated and very little was resolubilized. The concentrations in Table 3 reflect only the solubilized protein. The internal fragments were expressed in the range of 2-3.6 mg/ml. It was extremely difficult to determine the concentration of fragment 105-168 (it was suspected that the concentration was much higher than indicated), as the protein bands on the SDS gel were large, but poorly stained. The

negative control contained several background proteins as expected, but no obviously induced dominant protein.

Example 12 - Induction of HR in Tobacco

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The full length positive control protein elicited HR down to only 5-7 µg/ml. The negative control (pET 28) imidazole purified "protein" - which contained only background proteins - elicited an HR response down to the 1:2 dilution, which lowered the sensitivity of the assay as the 1:1 and 1:2 dilutions could not be used. This false HR was likely due to an affinity of the imidazole used in the purification process to bind to one or several of the background proteins, thereby not completely dialyzing out. Imidazole at a concentration of ca. 60 mM did elicit a false HR response.

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One definitive domain encompassing a small internal region of the protein from a.a. 137-180 (SEQ. ID. No. 23), a mere 44 a.a, is identified as the smallest HR domain. The other potential HR domain is thought to be located in the N-terminus of the protein from a.a. 1-104 (possibly a.a. 1-75) (SEQ. ID. No. 23). It was difficult to confirm or narrow down the N-terminus HR domain due to the difficulties encountered in purifying these fragment proteins. The N-terminus fragment proteins had to be purified with urea as no protein was recovered when the native purification process was used. Consequently, these proteins precipitated during the renaturing process and were difficult or nearly impossible to get back into solution, thereby making it hard to run the proteins through the HR assay, as only soluble protein is able to elicit HR. Difficulty narrowing the N-terminus HR domain was only compounded by the fact that the negative control elicited false HR at the low dilution levels thereby reducing the sensitivity of the assay.

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Surprisingly, when the internal HR domain was cleaved between a.a. 168 and 169 (fragments 76-168 and 105-168) (SEQ. ID. No. 23) the fragment lost its HR activity. This suggests that the HR activity of fragment 1-168 (SEQ. ID. No. 23) should not be attributed to the internal HR domain, but rather to some other domain, leading to the assumption that there was likely a second HR domain to be found in the N-terminal region of the protein. However, as discussed earlier it was difficult to confirm this assumption.

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Sub A19

The hypersensitive response elicitor C-terminus (a.a. 210-403 (SEQ. ID. No. 23)) did not contain an HR domain. It did not elicit HR at a detectable level using the current HR assay. Even the large C-terminal fragment from a.a. 169-403 (SEQ. ID. No. 23) did not elicit HR even though it contained part of the internal HR domain. As stated above, cleaving the protein between amino acids 168 and 169 (SEQ. ID. No. 23) causes a loss of HR activity.

Sub A20

Because some of the small cloned proteins with 61 a.a. or less were not expressed, several oligopeptides were synthesized with 30 a.a. to narrow down the functional region of the internal HR domain. The oligopeptides were synthesized within the range of a.a. 121-179 (SEQ. ID. No. 23). However, these oligos did not elicit HR. It was not expected that there would be an HR from oligos 137-166, 121-150, and 137-156 (SEQ. ID. No. 23) as these fragments did not contain the imperative amino acids 168 and 169 (SEQ. ID. No. 23). It was expected that the oligo 150-179 (SEQ. ID. No. 23) would elicit an HR. It is possible that 30 a.a. is too small for the protein to elicit any activity due to a lack of folding and, therefore, a lack of binding or that during the synthesis of the peptides important amino acids were missed (either in the process, or simply by the choice of which 30 amino acids to synthesize) and, therefore, the fragments would not be able to elicit HR.

Example 13 – Induction of Plant Growth Enhancement (PGE)

The C-terminal fragments enhanced the growth of tomato by 9% to 21%. The N-terminal fragments enhanced the growth of tomato by 4% to 13%. The internal fragments enhanced growth by 9% to 20%. The 76-209 fragment enhanced growth by 18% at a concentration of 60 µg/ml, but not at the typical 20 µg/ml. This was attributed to the inaccuracy of the quantification process (Table 4).

Table 4

Fragment #	Amino acids	PGE ht>buffer @ 10 µg/ml	PGE ht>buffer @ 20 µg/ml
1 (+ control)	1-403	12%	11%
2 (- control)	-	-3%	-2%
4	169-403	9%	12%
5	210-403	13%	14%
6	267-403	21%	16% @ 40µg/ml 21% 23% @ 40µg/ml
7	343-403	7%	7%
9	1-104	4%	8%
10	1-168	13%	5%
13	76-209	7%	4% 18% @ 60µg/ml
14	76-168	18%	20%
15	105-209	14%	19%
17	105-168	19%	16%
19	137-204	11%	13%
20	137-180	--	9%

*A height greater than 10% above the buffer control was necessary to pass the PGE assay.

The oligopeptides enhanced growth from 7.4% to 17.3% (Table 5).

Table 5

Fragment	Amino acids	Expression	HR titer	TMV efficacy	PGE ht>buffer
oligo	150-179	NA	-	72.9%	10.1%
oligo	137-166	NA	-	61.2%	12.0%
oligo	121-150	NA	-	60.0%	17.3%
oligo	137-156	NA	-	-87.7%	7.4%

The data suggests that there is more than one PGE domain, although the C-terminal and internal domains appear to be dominant over the N-terminal domain, as the N-terminal fragments enhanced growth the least amount.

Example 14 – Induction of Systemic Acquired Resistance (SAR)

All of the hypersensitive response elicitor fragments tested to date appear to have 60% efficacy or greater, except for the oligopeptide 137-156 (Tables 5 and 6).

Table 6

Fragment #	Amino acids	Efficacy of TMV control
1 (+ control)	1-403	84% & 72%
2 (- control)	-	40% & 31%
4	169-403	64% & 79%
5	210-403	77% and 78%
6	267-403	70% and 72%
9	1-104	82%
10	1-168	69%
13	76-209	44% and 84%
14	76-168	83% & 87%
15	105-209	57% and 67%
17	105-168	89%
19	137-204	89% & 77%
20	137-180	64% & 58%

These data suggest that there are multiple SAR domains within the protein.

Example 15 – Relationship Between HR, PGE, and SAR

It is clear that the hypersensitive response activity is separable from the plant growth enhancement activity. The C-terminal fragments clearly enhance the growth of tomato by ca. 20% at a concentration of only 20 µg/ml, but these same fragments were not able to elicit HR in tobacco, even at higher concentrations than 200 µg/ml. The SAR activity also appears to be separable from the HR activity. This finding is highly significant for future work on transgenic applications of the hypersensitive response elicitor technology. The fragments that induce PGE and/or SAR but do not elicit HR will be imperative for this technology, as constitutive expression of even low levels of an HR elicitor might kill a plant.

Example 16 - Non-HR Eliciting Fragments Derived from the Hypersensitive Response Elicitor from *Pseudomonas syringae* pv. *syringae* Induce Resistance in Tobacco to TMV and Promote the Growth of Tomato

To test whether non-HR eliciting fragments derived from HrpZ, the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, is able to induce disease resistance, several fragment constructs were made and the expressed

fragment proteins were tested for HR elicitation and disease resistance induction in tobacco and growth promotion in tomato.

The following segments of *hrpZ*, the gene encoding the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, were amplified by PCR using Pfu Turbo (Stratagene): Regions coding for amino acids 152-190, aa 152-294, aa 190-294, aa 301-341, and full length HrpZ (aa 1-341). The DNA fragments were cloned into pCAL-n (Stratagene) to create C-terminal fusion proteins to the calmodulin-binding peptide. pCAL-n was chosen, because the fusion protein could be easily and gently purified on calmodulin resin. The DNA was transformed into *E. coli* DH5 α , and the correct clones were identified. The clones were then transferred to *E. coli* BLR DE3 for protein expression. The bacteria were grown in Terrific Broth to an OD₆₂₀ of 0.8-1.0. Protein expression was then induced with IPTG and the bacteria were incubated for an additional 3 h. All of the HrpZ fragments were able to be expressed this way.

Amino acid fragments 152-294 and 190-294 were chosen for further analysis and characterization. It was expected that the fragment 152-294 contained a domain that elicited the HR, while fragment 190-294 contained no domain that elicited the HR. The cultures were spun down, and the bacteria resuspended in 40 ml of 10 mM Tris pH 8.0. Twenty μ l of antifoam and 40 μ l of 200 mM PMSF were added, and the bacteria was sonicated to break open the cells. The bacterial debris was removed by centrifugation, and the supernatant was placed in a boiling water bath for 10 min. The precipitate was removed by centrifugation and the supernatant, a crude protein preparation, was retained for tests.

Fifteen μ l of each supernatant was run on a gel and stained to determine if the protein was present. It was estimated that about five times as much of the 152-294 fragment was present as the 190-294 fragment. Several dilutions of each preparation were infiltrated into tobacco leaves on two plants for HR tests (Table 7). As shown in Table 7, the 152-294 fragment elicited an HR, but the 190-294 fragment did not.

Table 7

HR test results of HrpZ fragments

HrpZ Fragment	Dilution of Fragment Preparation ^a			
	1:2	1:5	1:25	1:125
152-294	+, ^b	+,+	+,+	-, -
190-294	-,-	-,-	-,-	-,-

^a The preparations were diluted with MilliQ water.

^b The results are indicated for each of two plants. +, HR; -, no HR.

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The fragment preparations were then tested for inducing resistance to TMV and for growth enhancement. Due to the difference in concentration of the HrpZ fragments, the 152-294 preparation was diluted 40-fold and the 190-294 preparation was diluted 8-fold. The results showed that the 190-294 aa fragment reduced the number of TMV lesions by 85% in comparison to buffer controls (Table 8). In contrast, the 152-294 aa fragment reduced the number of TMV lesions by only 55%. As also shown in Table 8, plants treated with the 152-294 aa fragment grew 4.64% more than buffer treated plants, while plants treated with the 190-294 aa fragment grew 2.62% more than the buffer treated plants.

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Table 8

HR test, TMV, and PGE test results

HrpZ Fragment	HR elicitation ^a	TMV (% efficacy) ^b	PGE(% > buffer ht) ^c
152-294	+	54.64	4.64
190-294	-	85.25	2.62

^a +, elicits HR in tobacco leaves; -, no HR in tobacco leaves.

^b % reduction in TMV lesions in unsprayed leaf of tobacco.

^c % greater height than buffer sprayed plants.

30

The results of these tests show that amino acids 152-190 appear to be involved in HR elicitation, because their removal eliminated the ability to elicit the HR. Both fragment preparations achieved disease control and growth enhancement. Thus, the ability to elicit the HR is not the determining factor for reduction in TMV infection and growth enhancement.

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Example 17 - Use of 13 Amino Acid Peptide Derived from *Phytophthora megasperma* Stimulates Tomato Seedling Growth

Parsley leaves develop a typical resistance reaction against the soybean pathogen *Phytophthora megasperma* comprising hypersensitive cell death, defense related gene activation, and phytoalexin formulation. Several years ago, a 42 kDa glycoprotein elicitor was purified from the fungal culture filtrate of *Phytophthora megasperma* (Parker et al., "An Extracellular Glycoprotein from *Phytophthora megasperma* f.sp. glycinea Elicits Phytoalexin Synthesis in Cultured Parsley Cells and Protoplasts," Mol. Plant Microbe Interact. 4:19-27 (1991), which is hereby incorporated by reference). Then, an oligopeptide of 13 amino acid was identified within the 42 kDa glycoprotein. The 13 amino acids peptide appeared to have similar biological activity as that of the full length glycoprotein (42 kDa). It is sufficient to elicit a complex defense response in parsley cells including H⁺/Ca²⁺ influxes, K⁺/Cl⁻ effluxes, active oxygen production, SAR gene induction, and phytoalexin compound accumulation (Nurnberger et al., "High Affinity Binding of a Fungal Oligopeptide Elicitor to Parsley Plasma Membranes Triggers Multiple Defense Response," Cell 78:449-460 (1994), which is hereby incorporated by reference).

To test if the 13 amino acid peptide derived from the 42 kDa protein also enhanced plant growth, 20 mg of the oligopeptide was synthesized from Biosynthesis Corp. The synthesized sequence of the peptide is NH₂-Val-Trp-Asn-Gln-Pro-Val-Arg-Gly-Phe-Lys-Val-Tyr-Glu-COOH (SEQ. ID. No. 39). The synthesized peptide was resuspended in 10 ml of 5 mM potassium phosphate buffer and, then, diluted to 1 and 100 ng/ml with the same buffer. About 100 tomato seeds (variety, Marglobe) were submerged in 20 ml of peptide solution overnight. The soaked seeds were planted in an 8 inch pot with artificial soil. Seeds soaked in the buffer without the peptide were used as a control. After seedlings emerged and the first two true leaves fully expanded, the height of the tomato seedlings was recorded. The peptide was not able to elicit the HR in tobacco and other tested plants. However, it had a profound effect on plant growth promotion. Table 9 shows that tomato seedlings treated with the peptide increased 12.6 % in height, indicating that the fungal peptide derived from the 42 kDa glycoprotein can promote tomato seedling growth. Extended studies showed that the peptide also had similar growth

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effect in other crops including tobacco. Similar growth promotion effects were achieved by plants sprayed with the peptide solution.

Table 9

5	Treatment	Height of seedlings (cm)					Average (cm) % Change	
10	Buffer	6.0	6.0	6.0	5.5	5.5	5.55	-
		5.5	5.5	5.0	5.0	5.5		
15	Peptide Solution (100ng/ml)	6.5	6.0	6.5	6.5	6.5	6.25	12.6
		6.0	6.0	6.0	6.0	6.5		

15 Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

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